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Brain Mapping-Based Model of Δ^9-Tetrahydrocannabinol Effects on Connectivity in the Pain Matrix

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Cannabinoids receive increasing interest as analgesic treatments. However, the clinical use of Δ^9-tetrahydrocannabinol (Δ^9-THC) has progressed with justified caution, which also owes to the incomplete mechanistic understanding of its analgesic effects, in particular its interference with the processing of sensory or affective components of pain. The present placebo-controlled crossover study therefore focused on the effects of 20 mg oral THC on the connectivity between brain areas of the pain matrix following experimental stimulation of trigeminal nocisensors in 15 non-addicted healthy volunteers. A general linear model (GLM) analysis identified reduced activations in the hippocampus and the anterior insula following THC administration. However, assessment of psychophysiological interaction (PPI) revealed that the effects of THC first consisted in a weakening of the interaction between the thalamus and the secondary somatosensory cortex (S2). From there, dynamic causal modeling (DCM) was employed to infer that THC attenuated the connections to the hippocampus and to the anterior insula, suggesting that the reduced activations in these regions are secondary to a reduction of the connectivity from somatosensory regions by THC. These findings may have consequences for the way THC effects are currently interpreted: as cannabinoids are increasingly considered in pain treatment, present results provide relevant information about how THC interferes with the affective component of pain. Specifically, the present experiment suggests that THC does not selectively affect limbic regions, but rather interferes with sensory processing which in turn reduces sensory-limbic connectivity, leading to deactivation of affective regions.
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INTRODUCTION

Exogenous cannabinoids are increasingly acknowledged as an alternative option for the treatment of pain. The major ingredient of *Cannabis sativa*, Δ^9-tetrahydrocannabinol (THC) (Mechoulam and Gaoni, 1965), has entered therapy of anorexia-associated weight loss (eg, in HIV), nausea and vomiting (eg, during chemotherapy), neuropathic pain in spastic paralysis (eg, in multiple sclerosis), and as an add-on to opioid pain treatment in cancer patients. However, its use for the treatment of pain (Farrell and Ritson, 2001) has progressed with justified caution (Kraft, 2012). This is owed to the risk of illicit use (Hall and Solowij, 1998) and also to the incomplete mechanistic understanding of its analgesic effects. It should be noted that studies in humans produced remarkably heterogeneous outcomes with respect to the effects of cannabis on pain, ranging from analgesia to hyperalgesia (Kraft, 2012; Walter *et al*, 2015b).

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Effects of cannabinoids on pain are supported by several lines of molecular and functional evidence. Cannabinoid CB_1 receptors (Devane *et al*, 1988; Matsuda *et al*, 1990) are ubiquitously present in the brain (Breivogel and Childers, 1998) and the spinal cord (Lindsey *et al*, 2005). Their activation may suppress the release of several neurotransmitters (Gulyas *et al*, 2004; Kano *et al*, 2009; Kofalvi *et al*, 2007; Piomelli, 2003), and thus regulate the function of several excitatory and inhibitory systems (Heifets and Castillo, 2009) such as glutamate, GABA, acetylcholine, and noradrenaline (Chevaleyre *et al*, 2006; Kano *et al*, 2009; Lovinger, 2008). CB_1 receptor activations have been associated with several perceptual and cognitive effects. They include an inhibition of chronic inflammatory and neuropathic pain (Agarwal *et al*, 2007; Bishay *et al*, 2010), a modulation of sensory information processing (Dervaux *et al*, 2013; Tart, 1970), often described as taking on new qualities, including nociception (Walker and Huang, 2002), a disruption of filtering of non-salient information (D'Souza *et al*, 2012) and attentional salience processing (Bhattacharyya *et al*, 2012; Solowij *et al*, 1991), and an extinction of aversive memories (Marsicano *et al*, 2002).

The complex effects of cannabinoids on information processing, shared among various functional entities throughout the brain, suggest that cannabinoids may

modulate pain perception by disturbing the connectivity within the human pain matrix (Apkarian *et al*, 2005; Bushnell *et al*, 1999; Price, 2000). As recently shown, THC reduces functional connectivity between the amygdala and the primary somatosensory cortex (S1) during pain processing, but it is not clear whether this effect results from reduced limbic output or reduced sensory input into the amygdala (Lee *et al*, 2013). Thus, the present study investigated effective connectivity of the pain matrix that is modulated by THC by applying dynamic causal modeling (DCM) and Bayesian model selection (BMS) to functional magnetic resonance imaging (fMRI) data collected during experimental pain stimulation. The results support the view that THC modulates effective connectivity in the somatosensory thalamocortical system, but additionally affects affective evaluation of pain by reducing sensory-limbic coupling.

MATERIALS AND METHODS

Subjects, Study Design, and Medications

The study (EudraCT-Nr. 2008-006881-27) followed the Declaration of Helsinki on Biomedical Research Involving Human Subjects and was approved by the Ethics Committee of the Medical Faculty of the Goethe University, Frankfurt am Main, Germany. Eight men (aged 24.9 ± 2.0 years (mean $\pm$ standard deviation), body weight 82.2 ± 7.0 kg) and seven women (28 ± 2.7 years, 64.7 ± 8.7 kg) were enrolled after having given informed written consent (Thirty-six subjects were scheduled for the whole study including six possible replacements subjects. Fifteen subjects underwent the present protocol while 15 other subjects underwent a modified protocol that will be analyzed in an independent context.). The subjects' health was ascertained via medical history, a short physical examination, and routine clinical laboratory tests. At the beginning of each study day, a urine drug screening for THC, opiates, cocaine metabolites, amphetamines at baseline (Mahsan-Kombi/DOA 4-Test, MAHSAN Diagnostika Vertriebsgesellschaft mbH, Reinbek, Germany) was performed to detect carry-over effects or illicit cannabis consumption. Before the experiments, medications except contraceptives, alcohol and food were prohibited for 1 month, 24 or 6 h, respectively. During the study days, subjects were not allowed to consume anything except water.

Employing a double-blind placebo-controlled randomized crossover design, subjects received either an oral dose of 20 mg THC (two capsules containing each 10 mg THC dissolved in Adeps solidus, manufactured by the hospital pharmacy of the University of Heidelberg, Germany) or placebo (Adeps solidus), with a washout interval of 24 days ($\pm$14 days). The succession of treatments was sex-matched and five men and three women received THC during the first study period. Measurements took place before (baseline) and 2 h post medication (THC or placebo) when maximum pharmacological effects were expected (Hollister *et al*, 1981). During measurements after THC administration, plasma concentrations of THC and its metabolites THC-OH and THC-COOH were 6.4 ± 3.9 ng/ml, 6 ± 3.2 ng/ml, and 43.3 ± 20.2 ng/ml (mean $\pm$ standard deviation), respectively (for further details, see Walter *et al*, 2013).

Experimental Pain and Stimulation Protocol

Pain was induced by trigeminal excitation, using a chemical stimulus (Kobal, 1981, 1985). Short stinging sensations were induced to the nasal mucosa by delivering short (500 ms) pulses of gaseous CO_2 to the subject's right nostril via a Teflon tube (outer diameter 4 mm). A concentration of 75% v/v ensured stimulation well above pain threshold (Oertel *et al*, 2012). To avoid mechanical or thermal co-stimulation, stimuli were embedded in a constantly flowing air stream (8 l/min) and applied via an olfactometer (Kobal, 1981; OM/2, Burghart Messtechnik GmbH, Wedel, Germany) that allowed for precise control of all parameters. CO_2 is converted into bicarbonate and protons (Tarun *et al*, 2003), which have been shown to excite trigeminal nociceptors via activating TRPV1 (Reeh and Kress, 2001) or TRPA1 (Wang *et al*, 2010) ion channels. This pain model is well established for clinical pharmacological pain research (eg, Kobal *et al*, 1990; Lötsch *et al*, 1998, 2006) including fMRI assessments (Oertel *et al*, 2008).

During each experimental session, 25 CO_2 stimuli were randomly interleaved with non-painful stimuli of 5 ppm H_2S or 0.8 ppm vanillin (olfactory stimulants) to reduce a contextual modulation of pain due to attentional bias toward nociceptive stimuli or pain expectancy (Tracey *et al*, 2002; Walter *et al*, 2015a). Stimuli were delivered at a randomly spaced inter-stimulus interval (ISI) of 13.5–28.2 s (mean 18.9 s), which was long enough to minimize habituation and adaptation processes (Hummel *et al*, 1994). Subjects rated the sensory perceptions on 100-mm visual analog scales (VAS) displayed randomly within 3.4–6.6 s (mean 4.9 s) after each stimulus and querying pain (0, 'no pain', to 100, 'pain experienced at maximum'), smell (0, 'no odor' to 100, 'odor perceived at maximum intensity') or pleasantness (0, 'very unpleasant' to 100, 'very pleasant', 50 mm indicating hedonically inert stimuli). However, only one out of these three ratings was queried randomly at a time to limit the duration of the experiments. In addition, after the completion of each fMRI measurement session, subjects rated 'fatigue', 'drowsiness', 'nausea', and 'euphoria' by means of VAS (length 100 mm, ranging from 'very weak' to 'very strong'), ie, at baseline and at the end of the post-drug session. VAS ratings were compared between experimental conditions by means of analysis of variance for repeated measures (rm-ANOVA; SPSS version 22, IBM SPSS Statistics, Chicago) using a 2×2 design with 'medication' (THC or placebo) and 'session' (baseline or 2 h post medication) as within subject factors. The α level was set at 0.05 and corrected for multiple testing according to the conservative criterion of Bonferroni (Hochberg, 1988).

fMRI Image Acquisition

Employing an event-related design (Friston *et al*, 1998), the blood-oxygenation level-dependent (BOLD; Ogawa *et al*, 1990) response to the pain stimuli was recorded on a 3T MR head scanner (Siemens Magnetom Allegra, Siemens Medical Solutions, Erlangen, Germany) equipped with a combined single channel transmit and 4-channel receive head coil. The subject's head was immobilized using foam pads. In each session, 750 volumes (32 slices, 3 mm thick, 1 mm inter-slice gap, descending order) were recorded using a T2*-weighted

gradient echo (GE) echo planar imaging (EPI) sequence (TR = 2048 ms, TE = 30 ms, flip angle = 90°, echo spacing = 420 μs, matrix size = 64 × 64, and in-plane resolution = 3 × 3 mm^2). Following volume acquisition, a magnetic field map was acquired for correction of image distortions due to magnetic field inhomogeneities (Andersson *et al*, 2001; Hutton *et al*, 2002) using GE imaging with identical geometric parameters and two different TE values (4.89 and 7.35 ms), from which magnitude images and a phase difference map were calculated. In addition, a high-resolution T_1-weighted anatomical image (1 mm isotropic resolution) was obtained for each subject via a three-dimensional (3D) magnetization prepared rapid acquisition of gradient echoes sequence (MP-RAGE; Mugler and Brookeman, 1991) using parameters TR = 2200 ms, TE = 3.93 ms, flip angle = 9°, TI = 900 ms, FOV = 256 × 256 mm^2 and one slab with 160 sagittal slices of 1 mm thickness, employing generalized autocalibrating partially parallel acquisitions (GRAPPA; Griswold *et al*, 2002) with an acceleration factor of 2 in phase encoding direction, yielding a duration of 4 min.

Data Analysis

Preprocessing of fMRI data. Spatial preprocessing of the MR data was performed using the statistical parametric mapping software SPM8 (Wellcome Department of Imaging Neuroscience, London, UK) (Friston *et al*, 1995; Worsley and Friston, 1995) on Matlab (version 8.3.0.532, MathWorks, Natick, MS). The first five volumes of each scanning block were discarded to ensure steady-state conditions. Volumes were realigned to the first volume and unwarped using the individual field map. The T1-weighted image was co-registered to the realigned and unwarped mean-EPI, segmented and normalized using 4th-degree B-spline interpolation (voxel size 3 × 3 × 3 mm^3). Spatial normalization parameters were applied to all EPI volumes, which were smoothed with an isotropic 9-mm FWHM Gaussian kernel.

Assessment of THC effects on the activity of the pain matrix. THC effects on the processing of nociceptive information in the brain were identified globally by applying a general linear model (GLM) aimed at identifying the components of the pain matrix showing altered activations following THC administration, which was followed by analyses of functional and effective connectivity aimed at identifying the sources of interactions in the functional network.

General linear modeling. The four scanning sessions acquired during the two study days were specified in one model. In the first-level analysis, the observed neurophysiological responses were partitioned into components of interest, confounds and errors. Each nociceptive stimulus was included as an event with zero duration. Events of no interest, ie, olfactory stimuli, visual requests for ratings, and subsequent button presses, were modeled as separate regressors within the design matrix but omitted from second-level analyses. Furthermore, the six rotational and translational parameters from the rigid body transformation obtained during image realignment were included as covariates of no interest. All regressors were convolved with the canonical hemodynamic response function (HRF). Low frequency fluctuations of the MR signal were removed by applying a high pass filter at 128 Hz. Voxelwise coefficients for all regressors were estimated using least squares analysis. Following model estimation, effects of interest were tested using linear contrasts to generate statistical parametric maps of *t*-values for each subject.

Second-level analysis employed a factorial 2 × 2 ANOVA design with factors 'medication' (placebo or THC) and 'session' (baseline and post medication) to calculate the contrasts for the two-way interaction terms. The resulting statistical parametric maps (SPMt) were interpreted with regard to the probabilistic behavior of Gaussian random fields. Results are reported at $p < 0.05$ (FWE-corrected) at peak position and a cluster size threshold of 5 voxels. The localization of brain activations, expressed in Montreal Neurological Institute (MNI) coordinates, was performed using the anatomy toolbox (version 2.5.2; Eickhoff *et al*, 2005) for those regions for which probability maps were available and the Talairach atlas for all other regions (Lancaster *et al*, 2000).

Psychophysiological interaction analysis. For analyses of psychophysiological interactions (PPI) (Friston *et al*, 1997), bi-linear models were used to identify brain regions that displayed THC-related changes in the correlations between the local activity and the activity in a distant seed region. The correlations were given by the mutual regression slopes. The ventral thalamus was chosen as the initial seed region based on its known role as a primary relay of sensory input to the cortex (Ab Aziz and Ahmad, 2006; Liang *et al*, 2011). The voxels from which activity *vs* time courses were extracted were taken from the main effect of 'stimulus' in the GLM analysis. An anatomical mask of the bilateral thalamus as region of interest (ROI) was created using the WFU PickAtlas (Maldjian *et al*, 2003) to identify peak activation in this region. A BOLD time course averaged across a 5-mm sphere centered on the peak coordinate of the group-level GLM main effect was extracted for each subject using the first eigenvariate of the time series at the volume of interest (VOI). Adjustment for the *F* contrast of the effects of the nociceptive stimuli served to remove other covariates (see above).

The time series of the BOLD signal for the seed VOI was de-convolved for each subject. This yielded the time series of the neuronal activity (Gitelman *et al*, 2003). The PPI interaction regressor was obtained as the scalar product of (i) the experimental context ('psychological variable') given from the main effects of pain stimuli applied either with or without THC and (ii) the time course of the neuronal activity at the seed region ('physiological variable'). The interaction regressor was forward-convolved with the HRF. PPI effects were estimated by identifying voxels that displayed differences in the regression slope that depended on THC. The resulting statistical parametric maps (SPMt) were submitted to a random-effect group analysis (one sample *t* test) and thresholded at $p = 0.05$, FWE-corrected.

Dynamic causal modeling. A dynamic causal model (DCM) (Friston *et al*, 2003) was built from the brain areas identified in the GLM and PPI analyses to infer the causal

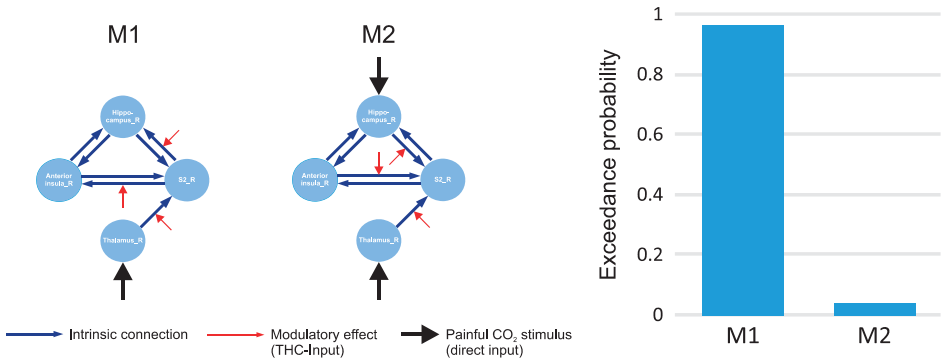

Figure 1 Left: Schematic structure of the two models compared in this study. In all models, seven intrinsic connections were defined (blue lines with arrows indicating the direction of connection). The driving input pain (black lines) enters the model via thalamus (model 1) and via thalamus and hippocampus (model 2), respectively. THC modulates the connection between thalamus and S2 (as known from the PPI analysis) and the connections from S2 to the anterior insula and to the hippocampus (model 1) or reverse, ie, from hippocampus and anterior insula to S2 (Model 2). Right: Result of Bayesian model selection on model level shows that exceedance probability of model 1 (EP: 0.96) exceeds model 2 by far (EP: 0.04).

architecture of the coupled dynamical systems involved in the generation of THC effects via Bayesian selection of the most likely model from a set of candidate models describing mutual influences of neuronal systems as differential equations (Friston *et al*, 2003). To estimate the effective connectivity between different brain regions, the brain was considered as a dynamic system driven by external perturbations (eg, experimental stimuli); and a hemodynamic model was applied that estimated hypothetical BOLD signals in such a way that they best reflected the measured BOLD signals. The accuracy of each model in describing the measured data was quantified by a negative free energy value called 'model evidence', which included a correction for model complexity. BMS was applied to obtain each model's probability to describe the observations relative to that of the other tested models.

Following prior evidence (Lee *et al*, 2013), we focused on the relationship between the limbic and the somatosensory components of the pain matrix. DCM was applied to the time courses extracted from four ROIs identified during GLM and PPI analyses, ie, the right ventral thalamus, secondary somatosensory cortex (S2), the hippocampus, and the anterior insula. As in the PPI analysis, a BOLD time course averaged across a 5-mm sphere centered on the peak coordinates of the PPI and GLM interaction effect was extracted using the first eigenvariate of the time series. Based on prior knowledge (Oshiro *et al*, 2009; Sim *et al*, 2006), bidirectional intrinsic connections between S2 and anterior insula, between S2 and hippocampus, and between anterior insula and hippocampus were assumed. We compared two different models regarding the modulation of connections by THC, which are based on the following considerations: one hypothesis is that THC diminishes flow of sensory information into limbic structures in the lateral spinothalamic pathway that projects from the thalamus to the somatosensory cerebral cortical areas and is forwarded to components of the limbic system. The other hypothesis is that THC reduces limbic modulation of somatosensory cortices. Therefore, two models were defined (Figure 1): The first model (Model 1) implied that (i) THC mainly influenced the processing of sensory information coming from the thalamus and (ii) additionally altered the connections from the secondary somatosensory cortex to regions involved in affective evaluations of pain (anterior insula and hippocampus). The competing second model (Model 2) implied that (i) THC mainly influenced the processing of sensory information coming from the thalamus and (ii) additionally altered connections from the anterior insula and the hippocampus to the secondary somatosensory cortex. The models were compared by means of random-effects (RFX) analysis (Stephan *et al*, 2010) applying Bayesian criteria to determine the best model. The winning model was further analyzed with respect to consistency across subjects by applying a one-sample *t*-test to the 'MAP' (maximum a posteriori) parameter estimates from the individual DCMs, separately for each parameter, ie, fixed connections, modulatory changes of connections and driving input.

RESULTS

All subjects finished the experiments, however, mild to moderate side effects occurred during the THC condition including an increase in the ratings for drowsiness, nausea, and euphoria (repeated-measures ANOVA: interaction 'medication' (THC, placebo) by 'session' (baseline, post-medication): all $F(1,14) > 4.94$, all $p < 0.044$), whereas fatigue was unaffected ($F(1,14) = 1.72$, $p = 0.21$). Further side effects during the THC condition comprised vomiting ($n = 2$), tremor ($n = 2$) and dizziness ($n = 2$). Neither THC nor its main metabolites were detected in any baseline sample.

Similarly to a previous experiment employing similar stimuli (Lötsch *et al*, 2012), the nociceptive stimuli were always perceived as painful (median [interquartile range]: 48.63 [33.19, 67.44] mm VAS pain) but not smelly (0 [0.0, 6.31] mm VAS smell), whereas the H_2S and vanillin were rated as smelly (31.44 [19.43, 47.5] and 22.06 [9.0, 31.9] mm VAS smell, respectively) but not painful (0 [0.0, 0.0] mm VAS pain). THC altered neither intensity perception (interaction 'drug' by 'measurement': $F(1,14) = 3.16$, $p = 0.097$) nor pleasantness rating (interaction 'drug' by 'measurement': $F(1,14) = 0.391$, $p = 0.542$) of the painful CO_2 stimuli. The intensity rating of the control stimuli H_2S and vanillin did not show any change, either ($p > 0.175$). For vanillin, however, THC reduced the hedonic rating from pleasant to neutral (64.58 and 52.53 for the baseline and

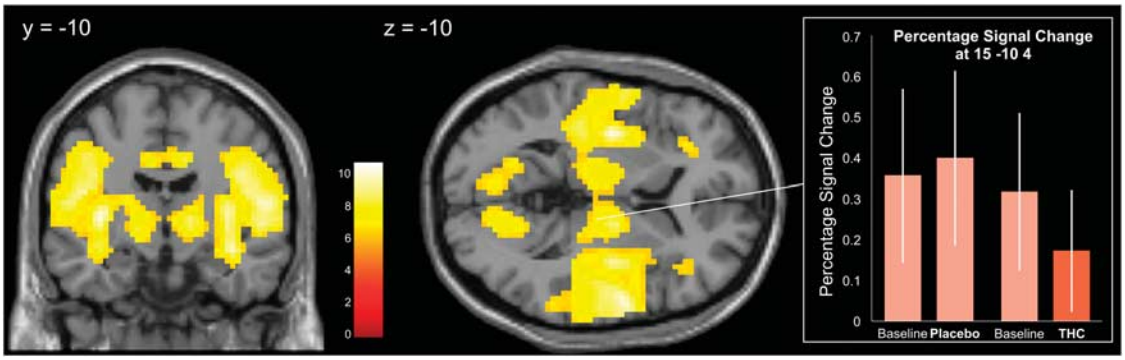

Figure 2 Brain regions that were activated by the CO_2 pain stimulus (main effect 'stimulus'). The topographies of differences in brain activations are superimposed upon slices of the canonical MR template implemented in SPM8. The significance at voxel level is color coded from red to white with increasing *t*-values. Voxels are shown at a threshold of $p<0.001$ (FWE-corrected, $t>6.18$). The bars show the effect size (mean and standard deviation) at coordinates of the right thalamus used as a seed region for subsequent PPI.

Table 1 Clusters of Brain Regions That Were Activated During the CO_2 Pain Stimulus (Main Effect 'Stimulus' in a 2 × 2 Factorial Design, Contrast 1 1 1 1 in the Succession Placebo Baseline Session, Placebo Post-Drug Session and THC Baseline Session, THC Post-Drug Session, Resp.)

Brain regions within cluster	MNI coordinates			t-values of peak coordinates
	x	*y*	*z*	
Right rolandic operculum/insula/ superior temporal gyrus	57	−4	10	10.73
Right anterior and median cingulate gyri/supplementary motor area	12	−16	37	9.35
Right calcarine fissure/left calcarine fissure/left cuneus	15	−73	7	8.03
Right postcentral gyrus	27	−28	52	7.74
Right cerebellum	18	−64	−26	6.86
Left cerebellum	−12	−64	−26	6.75
Left inferior frontal gyrus	−36	8	16	6.63
Right superior occipital gyrus	18	−82	28	6.58
Right thalamus[a]	15	−10	4	8.64

Results reflect a 14-subject analysis. Voxels are given at a threshold of $p<0.05$ (FWE-corrected, cluster size threshold 5 voxels). Coordinates are reported in MNI space (mm).
[a]Activity when applying an anatomical mask of the bilateral thalamus to identify region of interest (ROI) for connectivity analyses.

Table 2 Clusters of Brain Regions That Were Less Activated During the THC Condition in the Post-Drug Session (Interaction 'Drug' by 'Measurement' in a 2 × 2 Factorial Design, Contrast −1 1 1 −1 in the Succession Placebo Baseline Session, Placebo Post-Drug Session and THC Baseline Session, THC Post-Drug Session, Resp.)

Brain regions within cluster	MNI coordinates			t-values of peak coordinates
	x	*y*	*z*	
Right insula/inferior frontal gyrus	33	23	10	5.84
Right hippocampus/ parahippocampal gyrus	15	−7	−17	5.42
Left cerebellum	−27	−67	−20	4.56

Results reflect a 14-subject analysis. Voxels are given at a threshold of $p<0.05$ (FWE-corrected, cluster size threshold 5 voxels). Coordinates are reported in MNI space (mm).

post-THC session, respectively; interaction 'drug' by 'measurement': $F(1,14)=14.0$, $p=0.005$)), while H_2S remained unpleasant ($p=0.476$).

Whole Brain Analyses

CO_2 stimulus-induced brain activations were observed bilaterally, with slightly more pronounced activations in the right secondary somatosensory cortex, right postcentral gyrus (Figure 2, Table 1), which agrees with a previously observed right-hemisphere dominance of CO_2 stimulus-induced brain activations (Hari *et al*, 1997), and in addition in the cingulate gyrus. THC significantly reduced the activations in the right anterior insula, the hippocampus, and the cerebellum ($p<0.05$, FWE-corrected, SPM contrast −1 1 1 −1 in the succession placebo baseline, placebo post-drug session, THC baseline, and THC post-drug session; Table 2 and Figure 3). Enhanced activity following THC administration was not found (no significant activation at $p<0.001$, uncorrected, SPM contrast 1 −1 −1 1).

ROI in Functional Imaging

Psychophysiological interaction. The seed region for the PPI analysis was located in the right ventral thalamus at MNI coordinates $x=15$, $y=-10$, $z=4$, which had been identified by an ROI analysis of the bilateral thalamus as showing local peak activation within this region during pain stimulation (main effect 'stimulus'). The most pronounced PPI for this seed region was found with the right secondary somatosensory cortex (S2) at coordinates $x=45$, $y=-13$, $z=16$ (peak-level $p<0.05$, FWE corrected; Table 3 and Figure 4). THC induced a decrease in the connectivity between the thalamus and S2. This was supported by a tendency toward lower

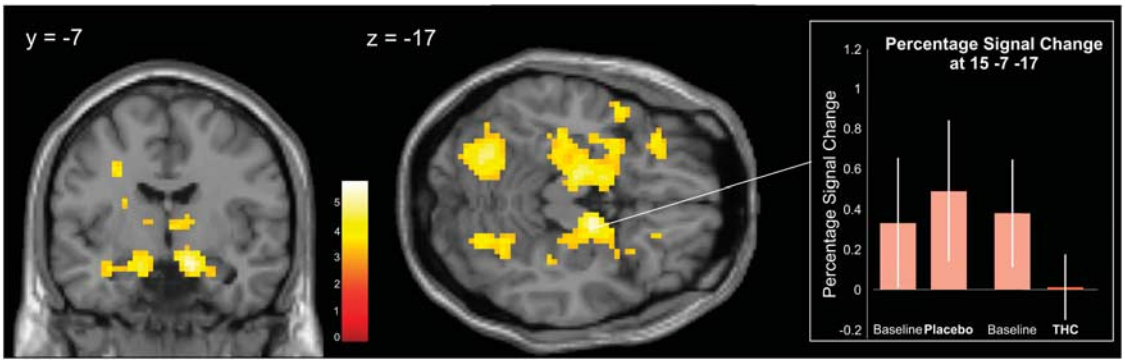

Figure 3 Brain regions that were deactivated by THC administration (interaction 'drug', ie, placebo or THC) by 'measurement' (ie, baseline or post-drug session). The topographies of differences in brain activations are superimposed upon slices of the canonical MR template implemented in SPM8. The significance at voxel level is color coded from red to white with increasing *t*-values. Voxels are shown at a threshold of $p<0.001$ (uncorrected, $t>3.25$). The bars show the effect size (mean and standard deviation) at the coordinates of the right hippocampus used as seed region for subsequent PPI.

Table 3 Brain Regions That Showed Reduced Functional Connectivity to the Seed Region Right Thalamus ($x=15$, $y=-10$, $z=4$) after THC Administration (Seed Region × (non-THC condition − THC condition))

Brain regions within cluster	MNI coordinates			t-values of peak coordinates
	x	y	z	
Right rolandic operculum/Heschl gyrus/insula/postcentral gyrus/superior temporal gyrus/precentral gyrus[a]	45	−13	16	14.23
Right calcarine fissure/cuneus Left calcarine fissure/cuneus	−3	−79	16	11.12
Left postcentral gyrus	−63	−1	13	11.04
Left postcentral gyrus/insula/rolandic operculum/Heschl gyrus	−36	−13	16	10.45
Right putamen	36	−4	−5	10.09
Right supramarginal gyrus/Heschl gyrus/rolandic operculum	45	−25	19	9.73
Left Heschl gyrus/superior temporal gyrus/postcentral gyrus/rolandic operculum	−57	−13	10	8.84

Results reflect a 14-subject analysis. Voxels are given at a threshold of $p<0.05$ (FWE-corrected, cluster size threshold 5 voxels). Coordinates are reported in MNI space (mm).
[a]Peak coordinates of this cluster are used as region of interest (ROI) for dynamic causal modeling (DCM).

pain intensity following THC administration (repeated measures ANOVA: interaction 'medication' by 'session': $F(1,14)=3.16$, $p=0.097$). An additional analysis performed solely to accommodate a previous finding (Lee *et al*, 2013) verified a reduced connectivity also between the amygdala and primary somatosensory cortex (see Supplementary Materials).

Dynamic causal modeling. The analysis of effective brain connectivity focused on four regions that previously had been identified by means of GLM (hippocampus: $x=15$, $y=-7$, $z=-17$, anterior insula: $x=33$, $y=23$, $z=10$) and PPI analysis (thalamus: $x=15$, $y=-10$, $z=4$, S2: $x=45$, $y=-13$, $z=16$) as regions where THC influenced the nociceptive activation and connectivity, respectively. DCM analysis compared models that included the regions and the direction of their interconnections that were influenced by THC. BMS showed that THC influenced the forward connections, starting from S2 to the anterior insula and to the hippocampus (model 1). This model was much more likely (exceedance probability 0.96) than THC influences on the direct nociceptive input to limbic areas with subsequent influence on sensory areas (model 2, exceedance probability 0.04). The parameters of the winning model 1 (Figure 1) showed that the connection strengths between thalamus and S2 and from S2 to the anterior insula or to the hippocampus decreased significantly under THC influence (Table 4).

DISCUSSION

Although the observed cerebral effects were not associated with major behavioral consequences, they provide nevertheless a plausible and statistically supported explanation regarding the analgesic effects of THC observed in clinical settings such as chronic pain conditions. These effects were associated with a modulation of nociceptive thalamocortical connectivity. The model selection analysis considered controversial assumptions about cannabis-induced modulation of interactions between affective and sensory processing underlying pain perception. It clearly favored a modulation of the affective component of pain on the basis of reduced sensory input into the limbic system. Tentatively, this could answer the open question concerning the direction of THC-induced reduced functional connectivity between

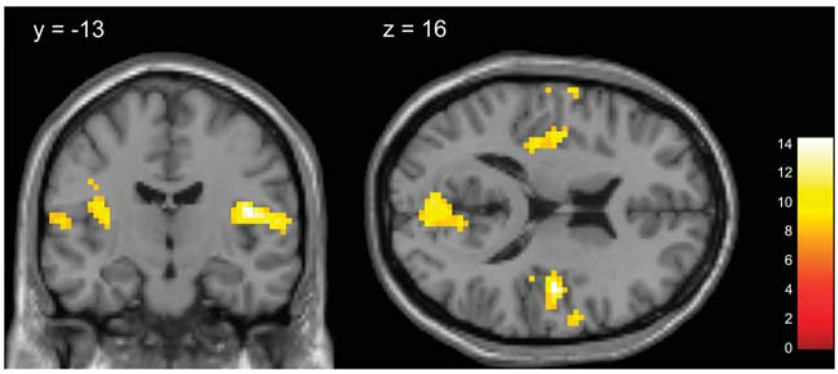

Figure 4 Brain regions that showed reduced functional connectivity to the seed region right thalamus ($x = 15$, $y = -10$, $z = 4$) after THC administration (PPI, seed region × (non-THC condition − THC condition)). The topographies of differences in brain activations are superimposed upon slices of the canonical MR template implemented in SPM8. The significance at voxel level is color coded from red to white with increasing *t*-values. Voxels are shown at a threshold of $p < 0.05$ (FWE-corrected, $t > 7.94$).

Table 4 Connectivity Estimates from the Winning Model 1

Parameter	Mean ± SD (Hz)	*t*-test (*t*; *p*)
Intrinsic connections (a parameter)		
Thalamus → S2	0.57 ± 0.46	$t(13) = 4.56$; $p = 0.001$
S2 → anterior insula	0.11 ± 0.32	$t(13) = 1.26$; $p = 0.229$
S2 → hippocampus	0.35 ± 0.64	$t(13) = 2.06$; $p = 0.06$
Anterior insula → hippocampus	−0.39 ± 0.72	$t(13) = -2.03$; $p = 0.064$
Anterior insula → S2	−0.18 ± 0.96	$t(13) = -0.72$; $p = 0.483$
Hippocampus → S2	−0.19 ± 0.69	$t(13) = -1.05$; $p = 0.31$
Hippocampus → anterior insula	0.01 ± 0.46	$t(13) = 0.07$; $p = 0.949$
THC effect on connections (b parameter)		
Thalamus → S2	−0.81 ± 0.76	$t(13) = -3.97$; $p = 0.002$
S2 → anterior insula	−0.41 ± 0.59	$t(13) = -2.59$; $p = 0.022$
S2 → hippocampus	−0.29 ± 0.5	$t(13) = -2.2$; $p = 0.046$
Direct input pain (c parameter)		
Thalamus	0.3 ± 0.29	$t(13) = 3.87$; $p = 0.002$

Results are given as means (± standard deviation) and *t*-values followed by the number of degrees of freedom in parentheses and the *p*-value. A two-sided one-sample *t*-test against zero was performed.

amygdala and primary somatosensory cortex (Lee *et al*, 2013).

The observation of deactivated limbic regions is in line with the observation that THC predominantly influences affective rather than sensory processing of nociceptive information. For example, administration of 5 mg oral THC in combination with 0.02 mg/kg intravenous morphine produced analgesic effects only on the affective but not on the sensory component of pain during heat stimulation (Roberts *et al*, 2006). Similarly, oral administration of 15 mg THC reduced the affective ratings of heat pain stimuli (Lee *et al*, 2013). The notion that THC influences predominantly the affective components of pain is further corroborated by THC's reported efficacy in chronic pain conditions (Abrams *et al*, 2007; Berman *et al*, 2004; Noyes *et al*, 1975; Nurmikko *et al*, 2007; Svendsen *et al*, 2004), especially when a psychological component such as pronounced distress is involved (Fernandez and Turk, 1992; Martin and Lichtman, 1998).

The identified brain regions, ie anterior insular cortex and hippocampus, correspond well with the assumption that THC effects on pain have a profound affective component. The (anterior) insula is involved in the identification of salient stimuli from a stream of sensory stimuli. It participates in most cognitive activities (Gasquoine, 2014), and has been proposed as an integral hub mediating the flow of information across brain regions (Menon and Uddin, 2010) via its bidirectional connections with most parts of the brain. The hippocampus, on the other hand, has been associated with novelty detection during acute painful stimulation (Bingel *et al*, 2002; Schneider *et al*, 2001). Hippocampal activity was reduced when attention was directed away from the painful stimulus (Ploghaus *et al*, 2000), and it was most consistently observed in studies in

which pain perception was increased by expectations or anxiety rather than by augmented nociceptive input (Leknes and Tracey, 2007). THC effects on the hippocampus are also in line with observations that cannabis contributes to the extinction of aversive memories to noxious stimuli (Marsicano *et al*, 2002).

However, cannabinoids have been associated not only with effects on the affective but also, although more rarely, with the sensory component of pain. Specifically in mouse experiments, THC significantly decreased the response latencies to heat stimuli (hot plate, tail flick; Martin and Lichtman, 1998). This was interpreted as indicating anti-nociceptive effects on a sensory level (Bloom and Dewey, 1978; Chesher *et al*, 1973), which would further agree with cannabinoid receptors being present in pain circuits from the peripheral sensory nerve endings up to the brain (Manzanares *et al*, 2006). Endocannabinoids are also involved in endogenous pain inhibition (Walker *et al*, 2001), as shown in models of chronic inflammatory and neuropathic pain (Agarwal *et al*, 2007; Bishay *et al*, 2010). Local injections of cannabinoid receptor agonists reduce pain in various rodent models (Agarwal *et al*, 2007; Kehl *et al*, 2003; Lozano-Ondoua *et al*, 2010). A few human studies also demonstrated a decreased sensitivity and increased tolerance to pain in a THC dose-dependent manner (Cooper *et al*, 2013; Greenwald and Stitzer, 2000), which is in line with the observation of reduced coupling between thalamus and S2 as the lateral spinothalamic tract is critical for the sensory-discriminative processing of pain (Maihofner *et al*, 2006; Price, 2002). The fact that subjects tended to perceive CO_2 stimuli as less intense during THC administration would support this effect. A covariation analysis using the pain ratings to analyze interindividual variability on neuronal effects was impossible because the perceived pain was queried alternatively with smell and pleasantness and therefore not available for each stimulus. Of note, the differences between the ratings of pain intensity and pleasantness of the CO_2 stimuli acquired after THC administration and at baseline did not correlate with the respective changes in the ratings of adverse effects, ie, drowsiness, nausea, euphoria, and fatigue, as indicated by always non-significant correlations (p-values always >0.05, Spearman's ρ never exceeding a low value of 0.26).

A recent imaging study demonstrated a THC-induced reduced functional connectivity between the amygdala and S1 in an experimental model inducing pain by means of capsaicin application (Lee *et al*, 2013). This effect could be reproduced in the present data (see Supplementary Material). However, such a conclusion, ie, that THC predominantly affects the limbic rather than the sensory processing of nociceptive information, was probably premature as with the exclusively used PPI method, the direction of the influence could not be identified.

To explore the influence of THC on connectivity between brain areas, we performed a PPI analysis to identify regions showing THC-induced altered connectivity with the ventral thalamus. No connectivity changes from thalamus to areas primarily processing affective components of pain could be identified although multiple ascending pain pathways transmit information from thalamus to the limbic system (Ab Aziz and Ahmad, 2006). Indeed, the PPI analysis identified S2 as the brain region showing the strongest reduction of functional connectivity with the thalamus upon THC administration. The activation detected in the present study is located at a minimally more anterior position than a previously shown hotspot of a thalamus-to-somatosensory cortex resting-state functional connectivity (Behrens *et al*, 2003). Given the observed PPI between the thalamic coordinate and the somatosensory cortex, the assumption that the present coordinates reflect the activity in thalamic sensory nuclei seems plausible. Furthermore, the results of the DCM analysis and subsequent BMS suggest that THC additionally modulated the information flow from the secondary somatosensory cortex to regions involved in the affective evaluation of pain rather than the opposite, ie, reducing the connection strength from limbic to somatosensory regions as previously interpreted from a PPI analysis (Lee *et al*, 2013).

Following the establishment of a plausible model of the THC effects on cerebral pain processing, a few further alternatives were additionally addressed: This first included two extreme alternatives to the present model, ie, a model that included direct pain inputs to the hippocampus and to the thalamus with separate influences of THC but without THC action on sensory-limbic connections (model 3; see Supplementary Material), and a further model that included THC effects on all intrinsic connections (model 4; see supplementary materials). These modifications did not change the results of the BMS, which still identified model 1 as clearly outperforming all other models ($EP>90$; details not shown). Moreover, the direction of influence from sensory to limbic areas also persisted when second, model comparisons were extended to two further models that were mainly identical to models 1 and 2 with the exception that both, incoming and outgoing limbic connections, were modulated by THC. Third, M1 is still favored when receiving the same direct input (ie in the thalamus and hippocampus) as M2. Fourth, as observations in laboratory animals suggested a direct THC influence on limbic regions, a model that included a direct influence of THC on hippocampus and anterior insula was considered in the BMS; however, it was assigned a probability of only 0.098. Of note, when analyzing only the baseline data acquired in the absence of any treatment, BMS also favored model 1 (EP: 0.91); however, in contrast to the model including THC data, the connection from thalamus to S2 was strengthened by the pain stimuli (b-parameter: 0.51 ± 0.56, $p=0.005$). Moreover, the parameters modulating the connection from S2 to hippocampus and anterior insula missed statistical significance (b-parameter: -0.05 and -0.09, respectively, with $p=0.77$ and 0.33, respectively), implying that a reduction of the transmission of nociceptive information to affective regions is consistent only in the presence of THC. Taken together, the exploration of several further models suggested by reported evidence or consisting in extreme or reasonable further alternatives did not result in any challenge of the present model.

Finally, reports of a THC-related increase in the brain perfusion, which subsequently caused fluctuations of the BOLD signal in several brain regions including the insula (Dodel *et al*, 2004; van Hell *et al*, 2011), point at a possible confounder that was not addressed in the present study as heart rate and respiration variability were not recorded. The referenced experiments were performed while subjects were in resting state, which has been highlighted to be particularly

vulnerable to misinterpreting artifacts as neuronal responses (Dodel *et al*, 2004).

CONCLUSIONS

In this work, we present THC effects on the cerebral processing of nociceptive information. THC modulates effective connectivity between the sensory thalamus and the secondary sensory cortex, but additionally reduces information flow from S2 to limbic regions. These findings have consequences for the way THC effects are currently interpreted: as cannabinoids are increasingly considered in pain treatment, present results provide relevant information about how THC interferes with the affective component of nociception. Specifically, THC does not selectively affect limbic regions, but rather interferes with sensory processing which in turn reduces sensory-limbic connectivity, leading to deactivation of affective regions.

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The authors declare no conflict of interest.

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